

Purification and Crystallization of Nitronate Monooxygenase: a Detoxifying Enzyme from *Williopsis saturnus*

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Nitronate monooxygenase (E.C. 1.13.12.16; NMO) is a detoxifying enzyme that oxidizes its substrate using a non-covalently linked cofactor, flavin mononucleotide (FMN). Substrate oxidation occurs through the formation of an anionic flavosemiquinone with oxygen as the final electron acceptor [1]. One oxygen atom is incorporated into the final product allowing for the classification of the enzyme as a monooxygenase. Propionate-3-nitronate (P3N) has been recently published to be the physiological substrate of NMO. P3N is the conjugate base of 3-nitropropionic acid and is highly toxic to succinate dehydrogenase and fumarase in the citric acid cycle. Inactivation of these essential enzymes by P3N leads to neurological disorders or death [2].

In this study, nitronate monooxygenase from *Williopsis saturnus* was expressed in *Escherichia coli* and purified to high levels through ammonium sulfate fractionation and two chromatographic steps. This procedure yielded an improved preparation with respect to that previously published in both specific activity and amount of enzyme. The purified enzyme was used to set up crystallization trials in order to obtain suitable crystals for X-ray crystallography. To set up protein crystals, nitronate monooxygenase was first concentrated through ultrafiltration and subjected to size exclusion column chromatography to remove free flavin from the enzyme solution. This study reports the current state of the project with an aim for the elucidation of the three dimensional structure of this important enzyme.

[1] Francis, K. (2005) *J. Biol. Chem.* **280**, 5195–5204.

[2] Francis, K. (2012) *Arch. Biochem. Biophys.* **521**, 84-89.

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